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APPLICANT(S): Kalle SAKSELA *et al.*

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FOR: METHODS AND MATERIALS FOR GENERATING SH3 DOMAINS WITH TAILORED BINDING PROPERTIES

DECLARATION SUBMITTED UNDER 37 C.F.R.§1.132

Honorable Commissioner of Patents
Washington, D.C. 20231

I, Dr. Mauno VIHINEN do hereby declare the following.

I am employed as a Professor of Bioinformatics in Institute of Medical Technology at Tampere University in Finland. One of the focus areas of my career has been molecular modelling of proteins, i.e. trying to understand their function and three-dimensional structures based on their amino acid sequence by computational analyses based on experimentally solved structures of related molecules. My research has specifically addressed modular protein-binding domains, including SH3 domains. I have published several articles related to these topics in high-ranking scientific journals and frequently discussed this subject matter in international symposia and congresses as an invited speaker.

Consequently, I believe that I am qualified to comment on two aspects of a concept that Dr. Saksela has termed "RRT-SH3 domains", namely the originality of this concept disclosed in the above-identified application and in the publication Hiipakka *et al.* 1999 (J. Mol. Biol. 293:1097-1106) in the light of his earlier work (Lee *et al.*, 1995, EMBO J. 14:5006-5015), and the applicability of this approach to SH3 domains other than Hck.

1) Originality of the RRT-SH3 approach in light of Lee et al.

In his earlier publication (Lee *et al.*, 1995) Dr. Saksela examined the differential capacity of two related SH3 domains to bind to the HIV-1 Nef protein. Based on comparison of Hck-SH3 and Fyn-SH3 amino acid sequences their attention was focused on a region in the

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SH3 structure known as the RT-loop. Such rational reasoning based on protein sequence data can be seen as a simple form of molecular modelling. By introducing Hck-like amino acid into the corresponding positions in the RT-loop of Fyn-SH3 domain, Saksela and colleagues could experimentally prove that this region indeed accounted for the differential binding to Nef, as hypothesized based on the sequence analysis.

The surprising results obtained in Saksela's later work (Hiipakka *et al.*, 1999) showing that entirely novel binding properties could be generated by random manipulation of this region of Hck-SH3 could not be envisioned based on the Lee *et al.* study. The approach taken in Hiipakka *et al.* was not rational design based on homology modelling and thereby conceptually opposite to that of Lee *et al.* The success of the RRT-SH3 domain approach (Hiipakka *et al.*) was particularly surprising to the scientific community, because despite the Lee *et al.* paper indicating a role for the residues in the tip of RT-loop in binding of Hck-SH3 to Nef, the contribution of this SH3 domain region to ligand binding had been considered to minor as compared with the extensively studied molecular contacts in the interface between SH3 domains and the proline-rich regions in their ligands.

In summary, in my mind the feasibility and success of Dr. Saksela's RRT-SH3 could not be envisioned based on the Lee *et al.* study.

2) Applicability of the RRT-SH3 approach to other SH3 domains

The general three-dimensional organization of all known SH3 domains is very similar. They all contain an RT-loop separating the first and second β-sheets of the SH3 fold. Similar to the Hck-SH3 domain, which Dr. Saksela has used as a scaffold for engineering RRT-SH3 domains, the RT-loops of other SH3 domains contain residues that contribute to the core structure of the domain as well as a flexible loop structure extending from this core. Although unexpected complications could of course occur in individual cases, overall it is reasonable to assume that the RRT-SH3 approach tested and proven using Hck-SH3 as a scaffold could be applied to most other SH3 domains found in the nature.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Tampere 02.03.2004

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